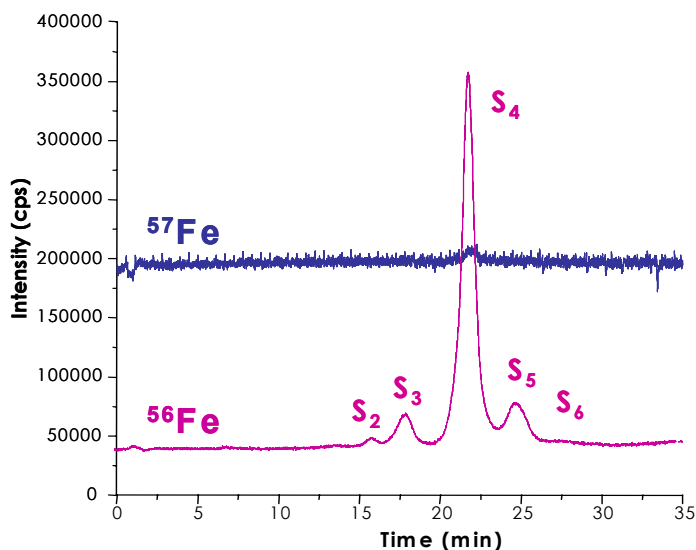


## Supplementary Electronic Material

### 1. Post column isotope dilution analysis.

1. Intensities chromatogram ( $^{57}\text{Fe}$  continuously introduced post-column and  $^{56}\text{Fe}$  mainly coming from the Fe present in the Tf).



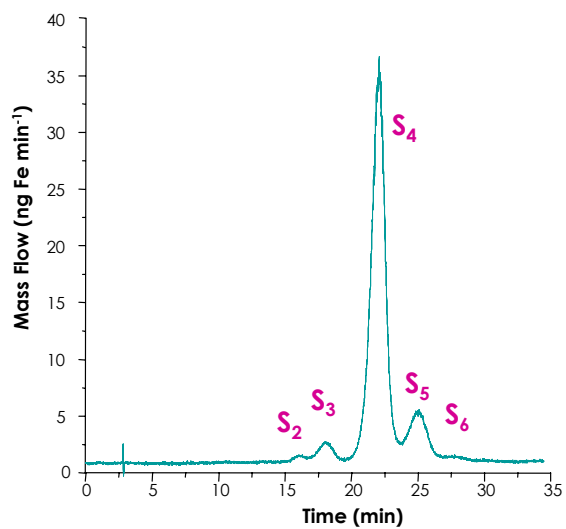
2. Correct for dead time and smoothing of the data at both masses. Then, the isotope ratio (in every point of the chromatogram) is calculated and corrected for mass bias.

3. The application of the IDA equation [1] to each ratio:

$$M_s = C_{sp} \cdot f_{sp} \cdot d_{sp} \cdot \frac{Aw_s}{Aw_{sp}} \cdot \frac{A_{sp}^{56}}{A_s^{56}} \cdot \frac{R_m - R_{sp}}{1 - R_m \cdot R_s}$$

All the parameters in the equation are known and only  $R_m$  is the measured isotope ratio in the chromatogram.  $R_s$  and  $R_{sp}$  are the ratios in the sample (natural) and in the spike (enriched  $^{57}\text{Fe}$ ).  $A_{sp}$  and  $A_s$  are abundances of  $^{56}\text{Fe}$  in the spike and the sample and  $Aw$  the atomic weight in the sample and spike respectively. Thus, for every  $R_m$  it is possible to obtain a  $M_s$  (mass of Fe) and to obtain the so-called mass flow chromatogram.

4. Mass Flow Chromatogram: represents the  $\text{ng Fe min}^{-1}$ . By integration of each peak, we can obtain the ng of Fe present on each isoform.



## **2. Isotope pattern deconvolution.**

As an illustrative example, we will show the calculation of Tf saturation of the tetrasialotransferrin (S<sub>4</sub>). The general equation is as follows:

$$\begin{pmatrix} I_{56} \\ I_{57} \end{pmatrix} = \begin{pmatrix} A_{Fe}^S & A_{Fe}^N \\ A_{Fe}^S & A_{Fe}^N \end{pmatrix} \cdot \begin{pmatrix} b_1 \\ b_2 \end{pmatrix}$$

The mid matrix corresponds to the abundances of natural Fe and Fe tracer. Thus, in this case is

$$\begin{array}{cc} \mathbf{Fe}_{\text{Natural}} & \mathbf{Fe}_{\text{Tracer}} \\ 56 & \begin{pmatrix} 0.04427 & 0.91754 \\ 0.95055 & 0.02119 \end{pmatrix} \end{array}$$

Isotope abundances of tracers are usually known directly from the analysis certificates and those of natural Fe can be obtained from IUPAC. <sup>54</sup>Fe and <sup>58</sup>Fe, very minor isotopes, are not monitored in this case.

The intensities of <sup>56</sup>Fe and <sup>57</sup>Fe obtained by HPLC-ICP-MS have to be converted into abundances according to the following equations:

$$A_{56} = \left( 1 - \frac{R_{57/56}}{1 + R_{57/56}} \right) \quad A_{57} = \left( \frac{R_{57/56}}{1 + R_{57/56}} \right)$$

where  $R_{57/56}$  is the measured isotope ratio in each point of the chromatogram corrected for mass bias. Therefore, in the maximum of the S<sub>4</sub> we obtained this numerical notation of [1]:

$$\begin{pmatrix} 0.28729 \\ 0.71270 \end{pmatrix} = \begin{pmatrix} 0.04427 & 0.91754 \\ 0.95055 & 0.02119 \end{pmatrix} \cdot \begin{pmatrix} b_1 \\ b_2 \end{pmatrix}$$

by solving this system,  $b_1=0.74359$  (tracer) and  $b_2= 0.27723$  natural, this means, that 27.72% corresponds to endogenous Fe and 74.35% to exogenous Fe.